Stem Cells in Translation

iPSCs in Clinics

Shinya Yamanaka
Kyoto University and Gladstone Institutes

Induced pluripotent stem cell (iPSC) technology has three major clinical applications in clinics: toxicology, drug screening, and regenerative medicine. Cardiac myocytes derived from SCs have already been used in pharmaceutical companies to predict cardiac toxicity of drug candidates. Similar approach can be applied to other types of cells derived from iPSCs.

In my opinion, the most promising application of iPSCs resides in drug screening. iPSCs reprogrammed from patients' somatic cells can be used to recapitulate disease processes. More than 100 papers reporting disease modeling using patient-derived iPSCs have been published in the last few years. These include not only monogenic diseases such as amyotrophic lateral sclerosis, but also polygenic diseases such as Alzheimer's disease. I expect that several effective drugs will be developed for currently intractable diseases within the next 10 years. Regenerative medicine is also promising.

Given the cost and time required to generate iPSCs under good manufacturing protocol (GMP), pursuing autologous transplantation may not be practical in the near future. Therefore, banking of iPSCs from HLA homozygous donors is an attractive alternative. A collection of 100 donors would sufficiently cover 50%–90% of patients, depending on ethnic groups. To effectively identify these donors, international collaborations are important, including the standardization of informed consent from donors and GMP regulations and the cooperation of existing medical banks such as bone marrow and cord blood banks.

Transplantation

Amy Wagers
Harvard University and HHMI

In the blood and skeletal muscle, lineage-specific stem cells maintain regenerative potential throughout life. These cells also provide a vehicle for cell replacement therapy in acute injury and degenerative disease. Indeed, transplant-based therapy has already been applied in the hematopoietic system; however, toxicity associated with pretransplant conditioning and post-transplant graft vs. host disease continues to restrict its application to only the most life-threatening conditions. In nonhematopoietic tissues, such as skeletal muscle, cell therapy approaches remain limited due, in part, to difficulties in isolating relevant regenerative populations and to challenges in the delivery of these cells, which do not home naturally via the bloodstream. Finally, identifying appropriately matched donors is a significant obstacle for transplantation in general, underscoring the need for new methods to derive "corrected" cells from autologous sources.

Yet although these challenges clearly represent substantial hurdles, recent discoveries are bringing game-changing insights and technologies. Most notably, remarkable advances in genome editing now promise the ability to specifically and selectively modify genomic sequences in patient stem cells to correct inborn mutations and to enable patients to serve as their own donors for autologous therapy. In addition, novel approaches to target stem cell niches, specify patient-specific regenerative cells from reprogrammed pluripotent cells, and engineer stem cell migration offer hope for innovative solutions to recalcitrant problems. These new horizons hold exciting potential for translating stem cell biology into new medical treatments.

Brain in a Dish

Alysson Muotri
University of California, San Diego

The unavailability of live human brain cells for research has blocked progress toward understanding mechanisms behind mental disorders. Genetic reprogramming offers a window on these disorders, as it captures a patient's genome in relevant cell types that can be propagated in vitro. Although still expensive and time consuming, this disease in a dish approach allows progressive time course analyses of target cells, offering an opportunity to reveal molecular or pathway alterations before symptomatic onset. Understanding current pitfalls of this model is crucial for correct data interpretation and for extrapolation of conclusions to the human brain. Creative strategies to collect biological material and clinical information from large patient cohorts are crucial to increase the statistical power that allows extraction of cellular phenotypes from the noise resulting from variability introduced by reprogramming and differentiation methods that affect phenotypic readouts. For example, through the "Tooth Fairy Project," we reprogram cells from baby teeth of autistic children. We interact with families by using social networks, which has dramatically increased the interest in this project over the years. Working with large patient cohorts is also important to understand how brain cells derived from the diverse human genetic background respond to specific drugs. Additionally, as several neurodevelopmental disorders share similar neurological symptoms, comparison of in-vitro-derived patient-specific neural cells provides insights into common trends and unique aspects of each neurological disease.

Cardiac Transdifferentiation

Deepak Srivastava
Gladstone Institutes

Cellular reprogramming approaches for cardiovascular (CV) disease hold great promise to transform our understanding and treatment of what remains the number one cause of death. The ability to reprogram adult cells into iPSCs that can be differentiated into large numbers of cardiomyocytes (CMs) represents a major technical advance. With this technology, our field is rapidly modeling myriad human CV diseases and then using these cells for drug discovery. Furthermore, human iPSC-CMs from a range of genotypes are being developed to screen for cardiotoxic effects of drugs, which account for ~60% of drug failure.

Use of iPSC-CMs for regenerative medicine will require additional technical advances, including efficient and consistent maturation, delivery, and integration. An alternative regenerative approach involves the direct reprogramming (transdifferentiation) of resident cardiac fibroblasts, which comprise more than 50% of cells in the human heart. We identified a combination of developmental cardiac transcription factors that reprogram adult cardiac fibroblasts toward a CM fate in vitro, without passing through a stem cell state. The same cocktail efficiently reprograms cardiac fibroblasts in vivo after cardiac injury in mice, resulting in new CMs that are mature, are electrically coupled, and improve cardiac function. Many questions remain regarding the mechanism and stochastic nature of direct reprogramming, but the paradigm of coaxing endogenous cells to adopt new fates for regenerative medicine represents an exciting new frontier for many cell types.

Stem Cell and Diabetes

Doug Melton
Harvard University and HHMI

Diabetes is a good example of a disease for which there is an obvious use for stem cells (SCs)—namely, making functional β cells to replace those lost (type 1 diabetes) or dysfunctional (type 2 diabetes). Although there are nontrivial complicating factors facing new treatments (immune rejection in T1D and insulin resistance in T2D), all diabetics would benefit from having more functional β cells.

The goal of making human β cells from pluripotent SCs is essentially at hand, and I believe that it will soon be possible to routinely make large numbers (billions) of glucose-sensing, insulin-secreting, human β cells. Transplantation of these cells could realize the dream of many insulin-dependent diabetics: to be relieved of the daily finger pricks, insulin injections, and debilitating complications of poorly controlled blood glucose levels. Then the principal challenges will be to find a method to block immune rejection of the transplanted cells and/or to develop an effective encapsulation device that protects the cells.

Finally, I think that a powerful new phase of SC science will come from combining different SC derivatives in immunocompromised mice. For example, imagine "reconstructing" human T1D by using a diabetic's iPSCs to make pancreatic β cells, immune cells (from human stem cells), and thymic epithelia and then transplanting all of these key cell types into a living test tube, the immunodeficient NSG mouse. Will the human disease develop in the same way in every mouse? Will iPSCs from different patients show a different developmental pathology? Using SCs to "reconstruct" human diseases may be our best chance to understand what causes degeneration and may point to new treatment avenues.

Cancer Stem Cells

Cédric Blanpain
Université Libre de Bruxelles

Many cancers have been shown to contain particular cells called cancer stem cells (CSCs), which display a higher ability to propagate tumor upon transplantation. However, this assay assesses the potential of the cells but not necessarily their actual fate during tumor growth. Lineage-tracing experiments have been recently developed to track the fate of tumor cells within their natural niche, lending to further support for the existence of CSCs.

There are, however, many questions that remain unanswered to exploit our basic knowledge of CSC for therapeutic applications. Are some markers specific for CSCs across different patients with the same tumors and among different cancers? Can we predict the clinical outcome of cancers based on the frequency and molecular signature of CSCs? Does elimination of CSC lead to tumor regression? What are the mechanisms that control renewal versus differentiation of CSC? Can we stimulate differentiation of CSCs or decrease their long-term self-renewing properties to achieve tumor regression in human cancers? Are the cells that sustain tumor growth the same cells that resist therapy and contribute to tumor relapse? What are the molecular mechanisms that mediate resistance of cancer cells to chemo and radiotherapy? Does targeting these mechanisms lead to a better, more complete, and long-lasting clinical response?

Clearly, more studies are needed to rigorously test the CSC hypothesis in different cancers and their importance in resistance to therapy. Addressing these open questions should provide new hints to better understand the mechanisms controlling CSCs and to develop new strategies to target these cells in clinical settings.

Secrets in the Egg

John Gurdon
Cambridge University

As development proceeds from embryo to adult, the differentiated state of cells becomes increasingly stable; fortunately it is very unusual for cells committed to one lineage to switch to another. Nevertheless a change to an embryonic state or to another kind of differentiation can be induced experimentally by somatic cell nuclear transfer, cell fusion, or transcription factor overexpression, as in iPSCs. However, such induced changes take place only at a very low frequency and are usually imperfect. I believe that a very important question asks by what mechanisms these induced changes take place. It seems very likely that reprogramming by nuclear transfer to eggs uses the same route as for sperm after fertilization. The sperm is a highly specialized cell, which is changed in a very short time to the entirely different male pronucleus, with 100% efficiency after normal fertilization. This depends on natural components of the egg. If we could identify these egg components and their mode of action, this information might be used to alter somatic cells before nuclear transfer so that their reprogramming by eggs to an embryonic state is much more efficient. It should then require less proliferation in vitro for the resulting embryonic stem cells, reducing errors that might occur and thus yield a higher quality of cells for replacement therapy in the clinic.

Power of Tissue Culture

Elaine Fuchs
The Rockefeller University and HHMI

In 1975, Howard Green and coworkers pioneered the culturing of human epidermal keratinocytes under conditions in which they could be maintained and passaged for hundreds of generations. They succeeded by realizing that epithelial cells rely upon mesenchymal factors for self-renewal and survival. Green also showed that, after long-term passage, epidermal stem cells from a patient could be cultured to restore skin over burned regions. These principles have been adapted and/or modified, most notably to grow corneal cells to treat blindness and to culture embryonic and intestinal stem cells. By parroting temporal environments experienced by embryonic cells as they diversify into tissues, the strategy is currently being exploited to achieve lineage-specific differentiation of iPSCs. There seems to be no end in sight to the variations on this theme. Will we soon be able to culture hair follicles, motor or dopaminergic neurons, cardiac muscle cells, or retinal cells for regenerative medicine purposes? Although these applications seem closer to our reach than ever before, the natural microenvironments that cells thrive in are not always easily recapitulated in vitro. Added hurdles are posed in coaxing progenitors that normally don't churn out tissue to do so. The Holy Grail to advance stem cell therapeutics now rests in the court of basic scientists who seek to unravel the crosstalk between stem cells, their niches, and their progeny.

Challenges Ahead

Rudolf Jaenisch
Whitehead Institute, MIT

Crucial technical challenges that stood in the way of using iPSCs for better understanding human disease have been resolved, including the generation of vector-free iPSCs, efficient gene-editing methods, and better-defined culture environments. Yet, I see four major hurdles that need to be overcome before the iPSC technology will be a standard approach for studying complex human diseases that will be used for routine clinical application.

(1) Though the developmental potential of iPSCs can be stringently defined in mouse, what are the most appropriate criteria to define quality of human iPSCs? (2) Another crucial issue is the choice of a control: because the differentiation potential between individual iPSC or ESC lines varies greatly, one cannot be sure that a subtle phenotype in a patient's iPSC is disease relevant rather than due to system immanent variation. The development of efficient gene-editing methods may allow the generation of isogenic pairs of disease-specific and control cells even for polygenic diseases. (3) Arguably the most pressing issue for producing a disease-relevant phenotype in the dish is how to efficiently induce iPSCs to generate self-renewing precursor cells such as hematopoietic stem cells and mature functional cells such as insulin-producing β cells. (4) Finally, are terminally differentiated or self-renewing precursor cells the most appropriate therapeutic cells in the routine clinical setting?

Given the breathtaking progress in the field, we can be optimistic that these technical hurdles will be resolved in the foreseeable future.